Conclusions.

The following conclusions may, I think, be justifiably drawn from these observations.

(1) More than one variety of (non-toxic) endsporing bacillus, resembling *B. tetani* in morphological characters, can be recovered from wound exudates in cases of the disease.

(2) There are at least three different types of (toxic) *B. tetani.*

(3) The "U.S.A. type" of the bacillus—that commonly used for the preparation of antitoxin—is not frequently obtained from wound exudates in cases of the disease occurring among men who have received prophylactic inoculations of antitetanic serum.

(4) Culture in a selective medium, followed by agglutination of the washed growth in presence of the three type sera, gives valuable information. It is, however, apparently not so delicate a test for the presence of *B. tetani* as is animal inoculation after culture of the wound exudate.

The Formation of Nitrites from Nitrates in Aqueous Solution by the Action of Sunlight, and the Assimilation of the Nitrites by Green Leaves in Sunlight.

By Benjamin Moore, D.Sc., F.R.S.

(Received October 12, 1917.)

(From the Department of Applied Physiology and Hygiene of the Medical Research Committee.)

The number of chemical changes brought about by the activity of light is multitudinous, and the study of these reactions has been very intensive in recent years. In the majority of the photo-chemical reactions, the effect produced is that of hastening an exothermic reaction, and in this resembles the action of a catalyst. The substances formed have a less content of chemical energy than the mother substances, and are usually of a more simple structural type. In such cases there is no clear proof of transformation, or conversion, of light-energy into chemical energy, and the light acts more as a detonator to a chemical reaction in which chemical energy is set free.

The most important case of an endothermic reaction set up by the action of light is that in which the synthesis of formaldehyde and carbohydrate is effected in the green leaf, by that action of light upon water and carbon dioxide in which the light-energy is converted into chemical energy and stored up.

It has been shown by Moore and Webster (1) that the first step, namely, that of formaldehyde formation, in which the greatest upbuilding of molecules with large storage of chemical energy occurs, can be effected by certain catalysts, such as the ferric and uranium salts in colloidal solution in water, when these are supplied with light-energy. Later, it was shown by Moore (2) that such inorganic catalysts are present in the chloroplasts of green cells.

If it be agreed that life at some period first arose on this or some other planet by a process of evolution from simpler constituents, it is clear that there must have arisen along the path of evolution some combination or mechanism for forming more complex molecules containing the elements carbon, hydrogen, oxygen and nitrogen, from simpler inorganic substances with a less content of energy. Otherwise, the substratum from which the living organism was to be built could not exist, and there would have been no store of existent chemical energy to act as nutriment for the simplest living organism and supply the essential energy for the acts of life.

The assumption that the green cell, with its complex structure and exceedingly highly organised chemical substances, such as the chlorophylls, formed at the dawn of life the first engine capable of utilising light-energy and producing a gain of chemical energy is entirely untenable, and would make a break in the continuity of inorganic and organic evolution, such as nowhere else is to be found.

It hence becomes of great importance to study fully the action of light upon those inorganic substances which are present in air and water, and might be presumed, from their nature and present position as nutrients of living organisms, to have been capable of being acted upon by light with inductance of endothermic chemical reactions, and formation of more complex compounds of organic character.

Our knowledge of the first steps in the assimilation of both carbon and nitrogen so as to take their part in the organic compounds is still very incomplete, but that regarding nitrogen assimilation is much the more fragmentary of the two.

It is true that the living cell can by linkage of reactions utilise the energy stored up in the form of carbohydrates, to induce endothermic reactions and build up fats and proteins. As has been pointed out by Moore (3), a certain amount of carbohydrates can be oxidised, and the energy so set free within the cell can be transferred to reduce another portion of carbohydrate to fat, or to reduce nitrogenous compounds and build in amino-groups to form proteins. Other examples of such linked reactions are seen in the action of certain bacteria and other unicellular organisms, such as Azotobacter, the nitro-bacteria of the nodules of the Leguminosæ, the philothionic organisms which derive stores of energy from the oxidation of sulphur or reduced sulphur compounds, and the iron bacteria which similarly utilise the energy obtained by oxidation of metallic iron or of ferrous compounds to build up organic carbon compounds from carbon dioxide and water. Such linked reactions require, however, the presence of a living cell containing protoplasm, possessing as its substratum organic compounds containing both carbon and nitrogen in very complex combinations. Moreover, the substrata of reduced compounds so utilised in linked reactions have demanded at earlier epochs the existence of living organisms for their reduction by the conversion of the energy of sunlight. None of these substances could have existed in a planet cooling down from a red-hot condition, on account of their chemical instability at higher temperatures. So also all the bound nitrogen in vegetable and animal organisms, and their decomposition products, such as coal, guano, and nitrates, must at one time have existed as atmospheric nitrogen, for no nitrates or nitro-compounds could have withstood the earlier high temperatures. The enormous stores of compounds containing the oxides of nitrogen now used in warfare, agriculture, and industry must have been formed endothermically from atmospheric nitrogen and oxygen with uptake of energy, and whether this occurred through the electric discharge of the thunderstorm or by the agency of living organisms, the first source of the energy, just as in the case of the organic carbon compounds, was the sun's rays. It follows that the agencies by which sunlight was utilised to form reduced compounds of carbon and nitrogen must have existed antecedently to the advent of life, for in its ultimate composition the substratum of proteins necessary to the living organism contains both types of endothermically produced radicles. It was such considerations which induced the series of experiments here recorded, which show that the energy of sunlight can be absorbed by dilute solutions of nitrates and institute an endothermic reaction in which the more reactive nitrites are formed even in absence of living organisms, and also that the green cells of plants possess the power of absorbing these nitrites.

It is well known from the thermo-chemical determinations of Faure, Thomsen, and Berthelot that in the formation of the oxides of nitrogen from their elements the acme of absorption of energy lies at the point of formation of nitric oxide (N_2O_2), and that the reaction runs endothermically towards this point, whether the starting point be nitrous oxide (N_2O) or nitrogen pentoxide (N_2O_5). The amounts of heat involved and differences in transition from one oxide to another are shown succinctly in the following Table abstracted from Mendeléeff (4), in which the numbers in the upper row represent thousands of gramme-calories for a gramme-molecular formation from the elements; and the lower shows in thousands of gramme-calories the heats of transition from one oxide to the other:—

N_2O		N_2O_2		N_2O_3		N_2O_4		N_2O_5
-21		-43		-22		-5		-1
	-22		$+21$		$+17$		$+4$	

$$\longrightarrow$$

This Table shows that in passage from N_2O_5 to N_2O_3, a supply of energy must be given to the reacting system amounting to $4+17 = 21$ thousands of gramme-calories for each gramme-molecule converted. The figures are for the gaseous condition, but it may be taken that they would be approximately the same for dilute solutions, and so that the amount of energy for the passage of a gramme molecule of a nitrate to a nitrite (say, from KNO_3 to KNO_2) would be about half this amount or about 10,000 grm. cal., for 1 grm. mol. of nitrogen pentoxide yields two of nitrate, which is converted into two of nitrite. These figures show clearly that the transition from nitrate to nitrite is a strongly endothermic reaction, and can only occur either by transformation of other forms of energy, such as that of light, into chemical energy, or by a linked chemical reaction with oxidation of previously formed reduced chemical substances.

Not only do the nitrites contain a greater storage of chemical energy than the nitrates, the energy potential factor of the energy quantity possesses a higher value, so that the nitrites react more readily than the nitrates, and many changes occur between living organisms and nitrites which are not given directly by nitrates. The experiments recorded below show that this endothermic reaction occurs in sunlight when dilute solutions of nitrates are exposed to the direct rays of the sun, either dissolved in redistilled water or as they occur in natural waters. In addition, it has been found that green leaves immersed in water possess, in presence of sunlight, the power of absorbing the nitrites so formed in the water.

In the earlier stages of the investigation the source of the nitrites obtained was not clearly understood. The outset point was that the nitrites and nitrates found to be present in atmospheric air by many previous observers could not be satisfactorily explained on the basis of the disruptive electrical discharges of thunderstorms, because there has not been traced any definite correspondence between the amount of nitrites and nitrates in air and rain-water and the prevalence of thunderstorms, such as must undoubtedly exist, were the energy of the lightning discharge the main cause of the production of nitrites and nitrates in the air.

Rain collected in the course of the present experiments when there had been no recent thunder was found to contain nearly as much nitrite (about 0·5 part per million) as rain caught in a thunderstorm, and, as has been shown by Ilosvay (5), the morning dew contains nitrites. Also, although the amounts of nitrites and nitrates present at any given time in the air are small, the amounts abstracted by condensing aqueous vapour and falling as rain or condensing as dew on the surfaces of leaves and ground in the course of the year is enormous, and this would appear to demand some uniformly distributed and more constantly acting source of energy, such as sunlight, rather than be dependent upon fortuitous electrical discharges.

These nitrites of the rain and dew form one of the chief supplies of nitrogenous nutrition for plants and animals supported by soil not artificially enriched with nitrogenous manure; the experiments given below indicate that there is also a probable aërial uptake of nitrites by the green leaves.

A source of much error and confusion in estimating the so-called "active" oxygen of air, rain, or dew by different observers at meteorological stations has been the use of test-papers, impregnated with starch and iodides, which were moistened and exposed to air and indicated, by the rapidity of development of a blue colour, the degree of "active" oxygen in air. This "active" oxygen was assumed to be present mainly as ozone or hydrogen peroxide without more proof, but this liberation of iodine from iodides is accomplished quite as readily by nitrogen tri- or tetr-oxide as by ozone or hydrogen peroxide. All the more recent researches (7) indicate that ozone and hydrogen peroxide are absent from the air at the earth's surface, and the important purifying and bleaching reactions hitherto ascribed to them must now be transferred to the intermediate oxides of nitrogen. At high dilutions, such as are shown below to occur under atmospheric conditions, the odours of ozone and that of the oxides of nitrogen are indistinguishable.

The present experiments show that air, rain, and dew invariably contain a mixture of nitrites and nitrates, and that on keeping the nitrites pass over into nitrates, but by insolation this process is reversed, and nitrites are formed from nitrates.

The test used was Ilosvay's modification of the diazo-reactions discovered by Griess, yielding compounds deeply coloured even at high dilutions; one of the best of these reactions for the purpose is that in which solutions of sulphanilic acid and α-naphthylamine in acetic acid are added to the water suspected of containing nitrites (8). This test is given only by nitrites, and not by either ozone or hydrogen peroxide.

Ilosvay (6) by the use of this test showed that the well-known reaction upon a paper impregnated with starch and potassium iodide often used to show the supposed presence of ozone in the atmosphere was really produced by nitrites, and demonstrated that at the earth's surface both ozone and hydrogen peroxide were normally absent. The same observer (5) found a strong reaction for nitrites in the morning dew on various leaves and grasses, and also adsorbed upon ignited sand and upon earth exposed wet to the atmosphere, and in water in absorption tubes through which air was drawn.

This test will clearly indicate, by the development of a pink colour, the presence of nitrites in a dilution of one in ten million. The test, when applied to the solutions exposed to sunlight, as described below, gave reactions indicating amounts of nitrite lying between two in a million and one in ten million.

These concentrations may appear at first sight infinitesimally low, but attention must be paid to the enormous areas in green leaves over the earth's surface which are exposed to the reaction. The strengths of solutions from which living organisms absorb essential constituents from their environments often belong to this order of concentration. The concentration of silicic acid in pond water, from which diatoms build up their siliceous skeletons, is of the same order of magnitude. A similar condition of affairs emerges if the assimilation of carbon compounds is considered, for all such assimilation depends on a concentration of only about three parts *by volume* of carbon dioxide in 10,000 of atmospheric air.

The concentration of carbon dioxide in the atmosphere of 3 parts in 10,000 by volume, small as it may appear to support all life upon the earth, looks at first sight enormous, compared to the concentrations at which silica is absorbed in plants, or to the concentrations of nitrites with which we are dealing in the present experiments ; but this arises entirely from the usual conventional mode of expression of the concentration in relative gaseous volumes in the atmosphere.

If the mass of carbon dioxide dissolved in water be expressed in relationship to the mass of water, the ratio drops to the same order of magnitude as obtains in the case of other essential constituents demanded for organic life, all of which, it must be remembered, including carbon, are synthesised from solutions and not in gaseous form. Thus, the absorption-coefficient of carbon dioxide between a system of air and water may be taken sufficiently accurately for these purposes as equivalent to unity, so that if an atmosphere containing 3 parts in 10,000 of carbon dioxide be brought into equilibrium with water, the aqueous solution will contain three volumes of gaseous carbon dioxide in 10,000 volumes of water. That is, in 10 litres of water there will be dissolved 3 c.c. of carbon dioxide. Now, since 44 grm. of carbon dioxide gas measure at normal temperature and pressure 22,000 c.c., this means that 3 c.c. of carbon dioxide weigh approximately 6 mgrm.; 6 mgrm. of carbon dioxide in 10 litres, therefore, represents a concentration of 6 parts in 10,000,000 parts of water. It is the carbon in the carbon dioxide which is utilised in formation of the organic carbon compounds of the plant, and this stands to carbon dioxide in the relationship of 12 to 44, so that the concentration of assimilable carbon becomes reduced to about 1 part by weight in 6,000,000 parts of water.

So far as nitrogen assimilation is concerned, the amount of nitrogen required by plants is not on the average more than about 5 per cent. of the required carbon assimilation, so that, other factors, such as coefficients of distribution between air and water, being taken as equal, a concentration of nitrites or nitrates in the atmosphere or cell-sap about equivalent to 1 part of nitrogen in 120,000,000 parts of water ought to supply sufficient for assimilative purposes.

In a series of twenty experiments it was shown that both rain and dew invariably contain nitrites, but these slowly diminish, so that water drawn from a reservoir by a service tap gives a quite negative result when tested by the diazo-reaction for nitrites. If, however, another portion of this same sample of water be exposed, either in the open or within a transparent quartz container, to sunlight or an artificial source of light rich in short wave-lengths,* in one or two hours a strong reaction is given for nitrites. In this manner, by testing water before and after exposure, the presence and relative amounts of nitrite and nitrate may be determined. Similar changes, only greatly diminished quantitatively, are obtained when the exposures are made in glass vessels, showing that it is the ultra-violet waves which are most potent. That this result is due to conversion of nitrates into nitrites, and not conversion of dissolved nitrogen and oxygen, is shown by the fact that it does not occur with distilled water holding air in solution; but if a small amount of potassium nitrate, say, one part by weight in 10,000 parts, be added, an intense reaction occurs on insolation. In several experiments it was shown that the presence of green leaves from different types of plants diminished the amount of nitrites present after exposure, as contrasted with control flasks alongside containing no green leaves.

---

* At the time the experiments on the action of rays from the quartz mercury arc light upon nitrates were made it was thought they were original, but since the paper was written two references have been discovered to experiments made by observers in France, who have approached the subject from quite a different standpoint. The first observer, M. Lombard, 'Comptes Rendus Acad. des Sciences,' vol. 150, p. 227 (1910), found that when tap-water or dilute solutions of nitrates were exposed to this source of light nitrites were formed. This was later confirmed by D. Berthelot and H. Gaudechon, 'Comptes Rendus Acad. des Sciences,' vol. 152, p. 522 (1911), but neither communication speaks of the importance in nature of this change, or shows it occurs with sunlight, or draws attention to the occurrence in green leaves, and the activation thereby produced. Attention is mainly directed to the cause of the sterilisation of water by exposure to ultra-violet light.

It is clear from these experiments that nitrates taken up by the rootlets of plants from the soil can be converted into the more reactive nitrites in the green leaf with absorption of solar energy; simultaneously, non-nitrogenous organic bodies are being built up in the same situation, which suggests that by interaction under the influence of light protein synthesis as well as carbohydrate may occur in the green leaf.

The presence of nitrites and nitrates in rain and dew indicates their occurrence in atmospheric air, and this was ultimately proven in a series of experiments which showed that the main portion of the oxidised nitrogen from air is found in water, after bubbling air through it, as nitrate and not as nitrite.

Great care is required in order to give a rigorous proof of this, because the condition of the absorbed substances from the air may be modified in the act of collection if light be not carefully excluded, and nitrate from the air be changed by insolation into nitrite. This fact first emerged from a series of experiments intended to study the relative amounts of nitrite in air by day and by night, when apparently the interesting result was obtained that there was practically no nitrite in night air, but a considerable amount in day air. Just then the effect of light in converting nitrates into nitrites was learnt, and a repetition of the experiment was made, using a blackened bottle with distilled water as absorbent. This distilled water had been twice distilled, and was so free from nitrate that it gave no Griess-Ilosvay reaction even after prolonged exposure to ultra-violet light. The result now obtained was that, whether the air were bubbled through by day or by night, only a very slight reaction for nitrites was obtained; but, on now exposing to sunlight this distilled water through which air had been bubbled in darkness, whether by day or by night, a strong reaction was obtained in each case, showing that oxidised nitrogen is present always in air both by day and by night.

It is not possible to conclude that the relative amounts of nitrate and nitrite in bubbled air give an indication of the relative amounts of the two oxides of nitrogen in the air; for if the absorption be attempted in the presence of light there will be a reduction to nitrite, and if in darkness, the great volume of oxygen simultaneously bubbled through may have oxidised nitrite to nitrate, so that there is a labile equilibrium between a given degree of light exposure and nitrates, nitrites, and oxygen.

Attempts are at present being made to follow up the earlier stages of nitrogen fixation from the atmosphere. The light of the sun in the upper strata of the atmosphere, where the ultra-violet has not yet been absorbed, must produce vast amounts of ozone, and these disappear as the earth's surface is approached; in so disappearing, the ozone may oxidise nitrogen and give nitrites and nitrates. Whatever their source, these nitrites are most reactive with organic substances and destructive to micro-organisms, and many of the natural bleaching, deodorising, and sterilising activities in air and water which have been hitherto ascribed to ozone and hydrogen peroxide are more probably due to nitrites.

Summary.

Dilute solutions of nitrates exposed either to sunlight or to a source of light rich in light-energy of short wave-length (such as light from mercury vapour arc enclosed in silica) undergo conversion of nitrate into nitrite.

There is an uptake of chemical energy in this reaction transformed from light-energy as in formation of organic carbon compounds in foliage leaves; it is to be added to the relatively small number of endothermic reactions known to be induced by light.

Interposition of a layer of glass between source of light and solution of nitrate greatly slows the reaction, showing that the most effective rays are those of short length.

When green leaves are immersed in nitrate solution, comparatively little nitrite accumulates, indicating that nitrites are rapidly absorbed by the green leaf. Nitrates taken up by plants from soil would, in presence of sunlight, be changed to nitrites, which are much more reactive than nitrates. This indicates that the early stages of synthesis of nitrogenous compounds are carried out in the green leaf and aided by sunlight.

Rain-water collected for considerable time contains no nitrites, all having been oxidised to nitrates, but if exposed to bright sunlight or ultra-violet light for a few hours a strong reaction for nitrites is always obtained.

Freshly collected rain-water or dew always contains a mixture of nitrites and nitrates, as shown by the nitrite test appearing without any previous treatment of the water and the great enhancement of this on exposure to strong illumination.

Air bubbled through nitrite- and nitrate-free distilled water gives a mixed reaction afterwards when the water is tested for nitrites and nitrates, showing presence of both forms of oxides of nitrogen in air.

There is no hydrogen peroxide or ozone in air at surface level. The fresh odour in open air, commonly referred to as "ozone," is probably nitrogen peroxide, which at high dilutions has the odour of ozone. The oxides of nitrogen are probably formed by the action of sunlight, rich in ultra-violet rays, in upper regions of the atmosphere upon air and aqueous vapour.

Attention is drawn to the importance of these actions of light in purification of air and water, and enrichment of soils and waters by this continuous supply of matter essential to organic growth, the energy of which, like that for upbuilding of non-nitrogenous organic compounds, comes from sunlight.

REFERENCES.

(1) Moore and Webster, 'Roy. Soc. Proc.,' B, vol. 87, p. 163 (1913).

(2) Moore, *ibid.*, 1914, p. 556.

(3) Moore, 'Recent Advances in Physiology and Bio-chemistry,' edited by Leonard Hill, p. 29, *et seq.*, and p. 131. Arnold, London, 1906.

(4) Mendeléeff, 'Principles of Chemistry,' translated by Kemensky and Pope, vol. 1, p. 287. Longmans, London, 1905.

(5) Ilosvay, 'Bull. Soc. Chim.' [3], vol. 2, p. 666.

(6) Ilosvay, 'Ann. Chem. Pharm.,' vol. 124, p. 1 ; vol. 186, p. 2.

(7) Hayhurst and Pring, 'Chem. Soc. Trans.,' vol. 97, Pt. I, p. 868 (1910) ; Pring, 'Roy. Soc. Proc.,' A, vol. 90, p. 204 (1910), and Strutt, 'Roy. Soc. Proc.,' A, vol. 94, p. 260 (1918).

(8) Sutton's 'Volumetric Analysis,' 10th edition, p. 470. Churchill, London, 1911.